

REVIEW

CLINICAL APPLICATIONS OF
THE CONTINUOUS FLOW BLOOD SEPARATOR
MACHINE

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SUMMARY

The NC1/IBM or Aminco Continuous Flow Blood Separator Machine is a safe apparatus for the selective removal or exchange of either packed red blood cells, leucocyte-rich or platelet-rich layers or plasma.

Abnormal fractions from any of these layers may be collected and discarded. Normal constituents may be collected for therapeutic uses.

The wide scope of its applications includes important uses in clinical immunology: temporary provision of good leucocytes or platelets; harvesting of immune leucocytes (preparation of transfer factor at up to 10 units per harvest); removal of cryo- or macro-globulins, immune complexes or blocking factors; replacement therapy for antibody or complement deficiencies. Examples are given of such uses together with some of the medical problems so far encountered.

INTRODUCTION

In this review the continuous flow centrifuge (CFC) is considered from three viewpoints: (A) its operation; (B) its uses in clinical immunology; and (C) its complications.

(A) OPERATION OF THE CONTINUOUS FLOW CENTRIFUGE

The CFC was a machine designed for the separation of blood into packed red cells, buffy layer and plasma in 1965 by the joint co-operation of the IBM Corporation and the National Cancer Institute, Bethesda, for leukaemia treatment. Since that time many units have shown that this machine is a safe instrument for use on humans (Freireich, Judson & Levin, 1965; Judson *et al.*, 1968; Mathé, Amiel & Schwarzenberg, 1971; Buckner *et al.*, 1969; Jones 1968; Powles *et al.*, 1971b).

Method

The machine consists of a centrifuge bowl and atraumatic peristaltic pumps. The donor's

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blood is drawn into the centrifuge bowl by these pumps and is separated into packed cells, buffy coat and plasma layers. From separate tubing parts these fractions are collected (or one fraction removed) and the rest returned to the patient through an indwelling cannula in the other arm.

Heparin is used as anticoagulant instead of acid-citrate-dextrose, as the latter produces many unpleasant and serious side effects. The sterile disposable assembly sets and bowl are primed with sterile heparinized electrolyte fluids. About 400 ml of fluid is contained in these tubings and bowl at any time.

In small children who have a blood volume of about 1000 ml it is advisable that part of the returning line should contain suitable cross-matched blood.

Equivolumic exchanges can be conveniently carried out so that any abnormal fraction in the blood may be removed and replaced with a normal blood component, e.g. packed cells for packed cells, or plasma for normal plasma. It is usual to maintain the flow rate through the machine at 40 ml/min, and to alter the centrifugal speed for collecting different leucocyte- or platelet-rich fractions. At the conclusion of the run, blood is displaced by air and the patient receives an extra 400 ml of the original priming fluid.

Two versions of the CFC are available—the IBM, which has facilities for simultaneous dual exchange through a common pump, and the Aminco Celltrifuge, which is the more economical model.

Safety

Machinery. Safety devices are incorporated into the machine which automatically stop the peristaltic pumps in the advent of serious air leak into the system, or venous spasm in the patient. When these peristaltic pumps stop, no blood is pumped back to the patient and none is withdrawn either. Suitable self-isolating transformers reduce electrical hazards.

Infection. In over 120 donor treatments no infection has occurred when sterile disposable tubing sets (Avon Medical) were used and the bowl assembly was autoclaved at 120°C for 30 min at 15 pounds per square inch. The use of Browne sterilizer control tubes and repeated microbiological checks during runs help to maintain the standard of asepsis.

The transmission of 'transfusion hepatitis' is a serious risk for all these units working with blood products. Therefore it is mandatory that all staff, patients and blood products are adequately and stringently screened for the hepatitis B (Australia A) antigen, at frequent intervals.

Bleeding. Despite effective anticoagulation in these patients during and after the run, no serious bleeding episodes have occurred. Patients with serious thrombocytopenia (platelet counts: 20,000–80,000 per cubic mm) have been safely treated without serious bleeding. Where platelet loss in such patients may be critical, it is essential to run the centrifuge at higher *g* force (centrifuge speeds of 1500 rev/min) to keep as many platelets as possible in the buffy layer, and to cover the patient with fresh platelet transfusions. However, in most patients it has not been necessary for protamine to be used as the morbidity from serious bleeding is less frequent than that of protamine. But in those in whom there is evidence of bleeding, such as from the gastrointestinal tract, due to hyperviscosity, it would be advisable to antagonize the heparin with protamine at the end of the 'run'.

Cell function. In repeated examination of the erythrocytes, leucocytes and platelets, there was no difference in the morphological appearances of these cells from centrifuge specimens as contrasted with the peripheral blood of these normal donors. ⁵¹Cr red cell survival studies

in dogs (Buckner, Eisel & Perry, 1968), and our own experience in humans, show no evidence of haemolysis. Neutrophil function as measured by the *Candida* killing test, hexose mono-phosphate shunt activity, or ingestion of *Staphylococcus albus* (Herzig, 1972; Goldstein, *et al.* 1971) showed no impaired function when compared with the peripheral blood of these donors.

However, we have found that the use of acid-citrate-dextrose as anticoagulant is associated with marked impairment of lymphocyte transformation to PHA, and allogeneic mitomycin C-treated normal lymphocytes, but this effect was not seen when heparin was used.

Comment. This apparatus has been used in many centres throughout the world, and in over 2000 patient treatments over the last 6 years has been found to be safe for use in humans. It provides a readily available and quick method of removing any abnormal constituents, without alteration of the patient's blood volume during the exchange. It already has been widely applied in medicine and Table 1 summarizes known uses. Opportunities for its further application in the treatment of other refractory conditions may become more apparent later.

TABLE 1. Potential and current uses of the cell separator

I. Red cell exchange	
	Severe anaemia; replacing excess plasma with packed erythrocytes
	Polycythaemia rubra vera; replacing excess erythrocytes with plasma
	Severe haemoglobinopathy, e.g. sickle cell anaemia
	Intravascular haemolysis; replacing abnormal erythrocytes with normal erythrocytes and/or plasma
II. Leukaphoresis	
	Obtaining normal leukocytes for transfusion into severely infected agranulocytic patients, or patients with chronic granulomatous disease or other serious phagocytic defects
	Obtaining leukaemia cells for active immunization
	Removing excessive lymphocytes in chronic lymphatic leukaemia
	Obtaining immune antitumour lymphocytes for homologous immunotherapy in patients with cancer, e.g. melanoma, choriocarcinoma, neuroblastoma, sarcoma
	Obtaining leucocytes for production of 'transfer factor' against bacterial, viral, fungal or tumour cells
III. Plasma exchange	
	Malignant paraproteinaemia with or without hyperviscosity features
	Cryoglobulinaemia
	Acquired hypogammaglobulinaemia
	Removing circulating 'blocking factors' during the phase of cancer cell dissemination
	Collecting immune antibodies, e.g. antitumour antibodies, Rhesus antibodies, autoimmune antibodies
	Removing 'selected' protein-bound poisons and toxins and replacing with unbound plasma proteins
	Hyperlipidaemia
	Temporarily supporting reversible hepatic failures following hepatotoxic drugs or transplants
	Temporarily supporting acute hypercalcaemia or thyrotoxic crises not responding to conventional therapy
	Disseminated intravascular coagulation, to remove promoting agents and restore normal fibrinogen metabolism
	Replacement therapy for complement deficiencies
IV. Thrombophoresis	
	Normal platelets may be collected for transfusion into thrombocytopenic patients; excess platelets may be removed from thrombocytotic patients

(B) APPLICATIONS IN CLINICAL IMMUNOLOGY

I. Red cell exchange

In severe autoimmune haemolytic anaemia due to antibodies, plasma exchange, efficient to some 10% of the plasma volume, can remove 35% of IgG or IgA and 70% of IgM. Red cell exchange can be used to temporarily replace the patient's erythrocytes, which may be more fragile than normal. This could also be useful, when a bone marrow graft has to be done against ABO-compatibility, e.g. an O graft into a B recipient. The CFC therefore now enables adults to benefit in the way that neonates with erythroblastosis foetalis have done for so many years.

II. Leukaphoresis

Methods. Most of the cell separators (CFC) in current use throughout the world, have been for collecting granulocytes from the buffy layer, or to obtain leukaemia cells for subsequent immunotherapy programmes. Leucocytes obtained contain three times more leucocytes than the conventional yield from a pint of blood. The range of yield by the CFC is between 3×10^9 and 6×10^{10} leucocytes from normal donors, but total cell counts as high as 10^{11} or 10^{12} may be obtained in 500 ml of buffy blood, from chronic myeloid or acute myeloid leukaemia patients. Despite the centrifugation of blood at either 700 or 1000 rev/min it is not possible to achieve complete separation of the various leucocyte portions (see Figs 1 and 2) as these cells are invariably contaminated with red cells and platelets. However, at low centrifugations, it is possible to obtain high yields of granulocytes, lymphocytes and monocytes (Fig. 1). About 80% of PMN may be more selectively obtained if buffy layered blood

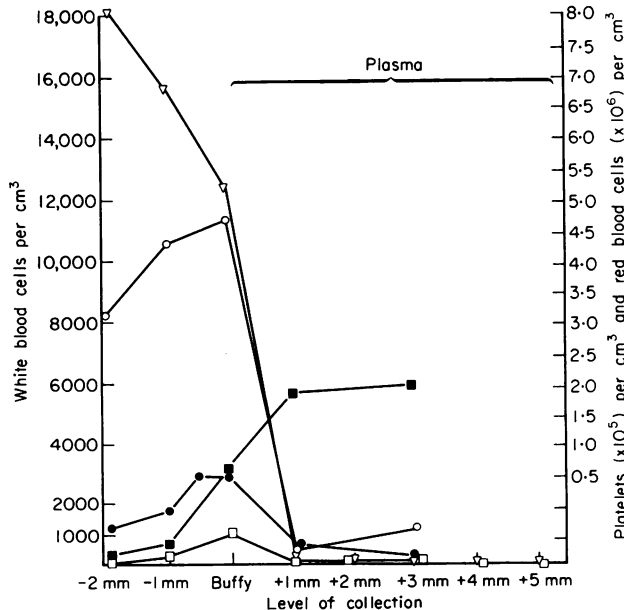


FIG. 1. Composition of cells at different levels of collection during leukaphoresis at 1000 rev/min. (∇) Red blood cells. (\circ) PMN. (\bullet) Lymphocytes. (\square) Monocytes. (\blacksquare) Platelets.

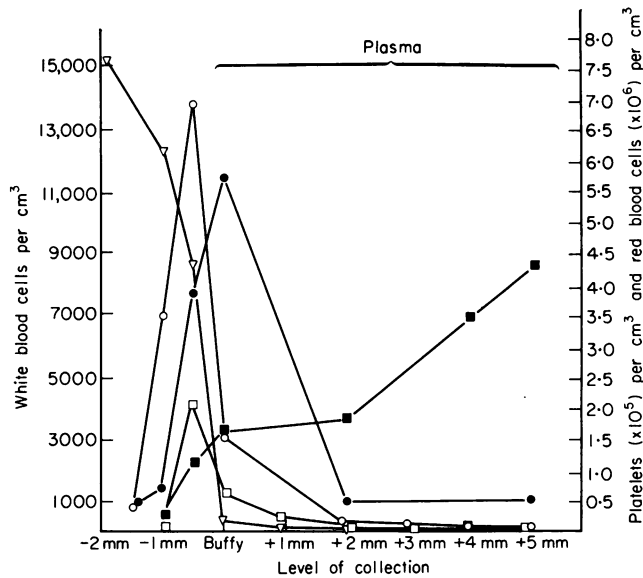


FIG. 2. Composition of cells at different levels of collection during leukapheresis at 700 rev/min. (▽) Red blood cells. (○) PMN. (●) Lymphocytes. (□) Monocytes. (■) Platelets.

is collected at 1000 rev/min (see Fig. 2). By using arteriovenous silastic shunts and a double centrifuge, Clift *et al.* (1973) were able to produce a yield of granulocytes far greater than a single centrifuge. McCreadie, Freireich & Hester (1973) used hydroxy starch to enhance red cell sedimentation and thereby a higher granulocyte yield, but the safety of hydroxy starch has not been proven for regular use.

Use of normal granulocytes. Severely infected neutropenic patients will benefit from the transfusion of normal granulocytes (PMN). Similarly in chronic granulomatous disease, and in acute myeloid leukaemias (Goldman & Th'ng, 1973), where bacterial and/or *Candida* killing are impaired, transfusion of granulocytes would be beneficial if these patients developed serious infections. Graw *et al.* (1972) in a study of seventy-six sequential neutropenic patients with Gram-negative septicaemia, found that when effective optimal antibacterial antibiotics were given to both the control group of thirty-seven patients (in whom suitable leucocyte donors were not available) and the transfused group of thirty-nine patients, 40% of the transfused group survived, as compared to 30% in the control group. Twelve patients who had four or more consecutive daily granulocyte transfusions from compatible donors survived.

Use of leucocytes as a source of transfer factor. 10^{10} leucocyte-rich buffy layer cells would provide at least 10 units of transfer factor, which can then be used for treatment in patients with impaired cellular immunity. Transfer factor has been found to be of value in the treatment of some patients with chronic mucocutaneous candidiasis (Valdimarsson *et al.*, 1972), generalized vaccinia, disseminated moniliasis, lepromatous leprosy and malignant diseases (Lawrence, 1969). It may be of possible value in Wiskott-Aldrich or Behcet's syndromes (Fudenberg *et al.*, 1974), or subacute sclerosing panencephalitis (Vandvik *et al.*, 1973).

Use in leukaemia. Leukaemic cells from patients with acute myeloid leukaemia can be antigenic and can induce immunological response in patients in remission (Powles *et al.*,

1971a). These leukaemia cells, may be stored, and at a later stage, used for immunotherapy programmes. The recent findings of Crowther *et al.* (1973) suggest that in patients having treatment by chemotherapy and immunotherapy (using BCG and irradiated allogeneic AML cells), survival and remission rates for these patients are longer than the chemotherapy group alone. Also Freeman *et al.* (1973) have found that patients on both immunotherapy and chemotherapy, can be more easily induced into remission than the chemotherapy group alone.

Mathé *et al.* (1971) have used normal allogeneic leucocytes in the treatment of acute leukaemias, and have produced remissions in a number of these patients. However, in the severely immunodepressed patients, there was an invariable graft versus host reaction, which was fatal in many of their patients. Fefer *et al.* (1974) have good evidence that, where an identical twin is available, leucocytes from such siblings may prolong remissions for over 100 days.

Objective evidence of regression in lymphadenopathy, spleen and liver sizes, bone marrow infiltration, were seen when patients with chronic lymphocytic leukaemia (CLL) were intensively leukaphoresed (Curtis, Hersch & Freireich, 1972). In a study of thirteen patients with CLL the median total of lymphocytes removed was 18×10^{11} (initial lymphocyte counts exceeded $20,500/\text{mm}^3$). Reich *et al.* (1971) tried the effect of massive leukaphoresis in patients with acute myeloid leukaemia (AML) by intensely leukaphoresing four of their patients. By removing 2.3×10^{11} to 3×10^{12} leukaemia cells from the peripheral blood, they reduced the original count by 15–44%. This produced a doubling in the mitotic index and the thymidine labelling of bone marrow blasts cells, which, it was felt, would be more vulnerable now to the effects of 'S' phase cytotoxic drugs. However, when thioguanine and cytosine arabinoside were used, the responses were identical for both the leukaphoresed and non-leukaphoresed groups of patients, suggesting that their patients were 'resistant' to such procedures.

Use of immune leucocytes as passive antitumour immunotherapy. Buffy-layer leucocytes harvested from immunized volunteers, given to the tumour donor, have produced remissions or regression in tumour-bearing patients (Nadler & Moore, 1966; Krementz *et al.*, 1974). For some of our neuroblastoma and osteogenic sarcoma patients, immune leucocytes present in the peripheral blood of the relatives were found to react more against the tumour cells (e.g. TI 88) of these patients, so they were harvested and used as treatment. Beneficial responses, though lasting only a short time, were seen in our neuroblastoma patients (Fig. 3). In our osteosarcoma patient who was given 'prophylactic' adriamycin followed by prophylactic immune maternal leucocytes, no tumour recurrence has been seen. She remains alive and well at 18 months follow-up. It is possible that this form of passive immunotherapy is only effective in the 'minimal residual disease' state, as the administered leucocytes are likely to be rejected within a week or so. In this respect it is important to be sure the recipient has a positive MLR. Some of these patients have converted from tuberculin negativity to positivity, suggesting that transfer factor may have been implicated.

III. Plasma exchanges

Choice of plasma preparations (see Table 2). The choice of suitable plasma preparations is important as there are differences between the different commercially available plasmas. Dry reconstituted plasma is made from the plasma of banked blood, from five to ten normal donors, all known to be Au antigen-negative. It contains higher potassium levels, is more acidic, and contains less clotting factors and complement. Fresh frozen plasma (FFP) is obtained from single donors (Au antigen-negative) and usually contains most of the clotting

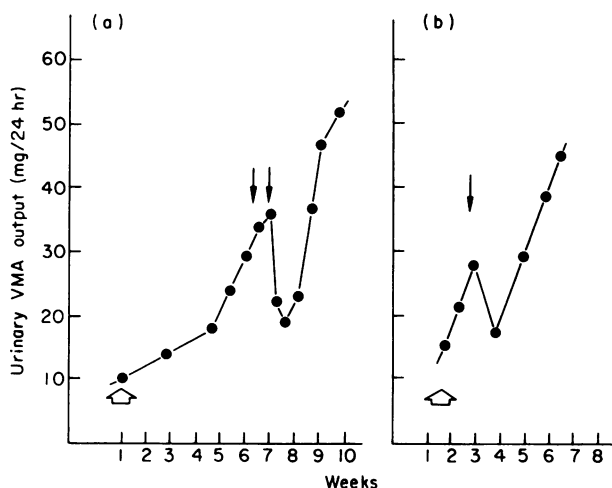


FIG. 3. Effects on 24-hr VMA output of administration of 10^9 – 10^{10} homologous immune leucocytes in two children with disseminated neuroblastoma. With the temporary falls, the VMA output doubles in 2–8 days. (a) Patient SB. (b) Patient DO. Large upward arrow signifies escape from DXR, vincristine and cyclophosphamide. The smaller downward arrow signifies infusion of homologous immune leucocytes.

serum factors. It is slightly alkaline and has to be carefully thawed out before use, as rapid thawing can damage the immunoglobulins. Both these preparations have low total calciums and they contain ACD. Human protein fractions are unsuitable for plasma exchanges as they contain no immunoglobulin, have a very high sodium content and very low calcium content. Thus, in patients with chronic renal failure who may have difficulty in excreting H^+ ions, the careful selection of the correct plasma is important. It is important also to bear in mind that (about 40%) patients with congenital IgA deficiency may have or develop anti-IgA antibodies. Such individuals may develop severe anaphylactic reactions when exposed to blood products containing IgA (Amman & Hong, 1971), and for these panels of IgA-deficient donors can be used.

Use in acquired hypogammaglobulinaemia. Patients with acquired hypogammaglobulinaemia require immunoglobulin replacements. Usual gamma-globulin preparations contain normal levels of IgG but are low in IgM and IgA (Medical Research Council Report, 1971). Table 2 shows that dry reconstituted plasma and FFP contains normal levels of these immunoglobulins. However, occasional bottles of FFP contain low levels of IgA.

Patients with hypogammaglobulinaemia secondary to a protein losing state are not effectively replaced by such treatment. A 45-year-old female with a 6-year history of chronic lymphatic leukaemia developed severe generalized hypoproteinaemia. Twenty-four-hour urinary protein loss was 40 mg, PVP did not show protein-losing enteropathy and despite very low serum albumin (2.3 gm/100 ml), globulins (3 gm/100 ml) were found. Hypoproteinaemic oedema developed. Plasma protein replacement by exchange was carried out, following which she had a copious diuresis and lost 2 kilos in weight. A 24-hr urine done after the exchange showed that she now had a massive non-selective proteinuria of 20 gm/day. Her hypoproteinaemia reappeared 48 hr later.

Patients 2 and 3. Two patients with thymoma and acquired generalized hypogamma-

TABLE 2. Composition of normal human parental plasma preparations: (a) dry reconstituted pooled plasma; (b) fresh frozen plasma; and (c) human plasma protein fraction

	Urea (mg %)	K ⁺ (mEq/l)	Na ⁺ (mEq/l)	HCO ₃ ²⁻ (mEq/l)	Ca ²⁺ (mg %)	PO ₄ ³⁻ (mg %)	Total protein (g %)	IgG* (mg %)	IgA* (mg %)	IgM* (mg %)	IgE* (i.u./ml)	Choles- terol (mg %)	Free thyroxine (µg %)	Serum iron (µg %)	TIBC (µg %)	pH†	Osmo- lality	Viscosity‡ of 25°C (units)
Normal ranges	20-40	3.5-4.5	135-145	20-30	9-10.5	2.3-4	5.5-7.5	560-1600	125-425	47-170	130-1830	130-330	4.5-11	60-150	250-400	7.35 7.34	—	155-180
Dry plasma	22	7.4	155	7	6.8	3.6	5.8	660	170	72	500	150	6.3	88	300	6.85	330	139
Au Ag- negative	22	6.7	156	6	7.2	3.8	5.8	660	170	72	600	250	7	88	—	7.0	335	149
ACD	23	7.3	158	6.5	7	—	5.6	560	170	70	500	100	4.5	80	—	6.9	330	151
Fresh frozen plasma	—	—	—	—	7.5	—	5.4	670	165	72	800	—	—	100	327	7.2	—	—
Au Ag- negative	—	—	—	—	7.4	4.1	5.6	—	—	—	—	—	—	—	—	7.13	—	—
ACD	23	4.1	135	11	6.9	3	5.7	640	150	92	360	180	3.6	109	336	7.63	280	153
Human plasma	19	4	138	13	7.2	2.8	5.6	560	(72)	48	420	195	3.9	105	300	7.5	280	145
protein fraction Au	20	3.8	137	11.5	7.3	2.9	5.7	840	105	92	590	—	—	—	—	7.60	274	155
Ag- negative	—	—	—	—	—	—	5.4	720	170	54	—	—	—	—	—	7.5	—	—
ACD	—	—	—	—	—	—	5.5	720	185	60	—	—	—	—	—	7.8	—	—
Human plasma	8	1.4	160	8	5	—	4.2	n.d.	n.d.	n.d.	n.d.	—	—	—	—	—	—	116
protein fraction Au	8	1.5	160	8	5	—	4.2	n.d.	n.d.	n.d.	n.d.	—	—	—	—	—	—	117
Ag- negative	8	1.5	160	8	5	—	4.0	n.d.	n.d.	n.d.	n.d.	—	—	—	—	—	—	116
ACD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

n.d. = Not detectable.

* Immunoglobulins by modified Mancini radial immunodiffusion.

† Measured by Astrup.

‡ Measured by Harkness Viscometer, Coulter Electronics.

globulinaemia, developed severe purulent bronchiectasis. One of them, a female aged 66 yr, developed a near fatal staphylococcal pneumonia. On recovery from this she has been maintained in good health by regular plasma replacements every 3 weeks. The second patient with this disease is a male, aged 70 yr, who has severe bronchiectasis and lichen planus. Both of these patients also suffer from mild ulcerative colitis and reacted severely to dry plasma preparations. The man was found to have multiple antileucocyte antibodies. Both these patients have not had any adverse reactions while having fresh frozen plasma (FFP), and clinically have done better (and gained weight) than when on γ -globulin injections.

Use in paraproteinaemia. Plasma exchanges have been performed in many units for the treatment of patients with excess paraproteins causing hyperviscosity symptoms. One patient

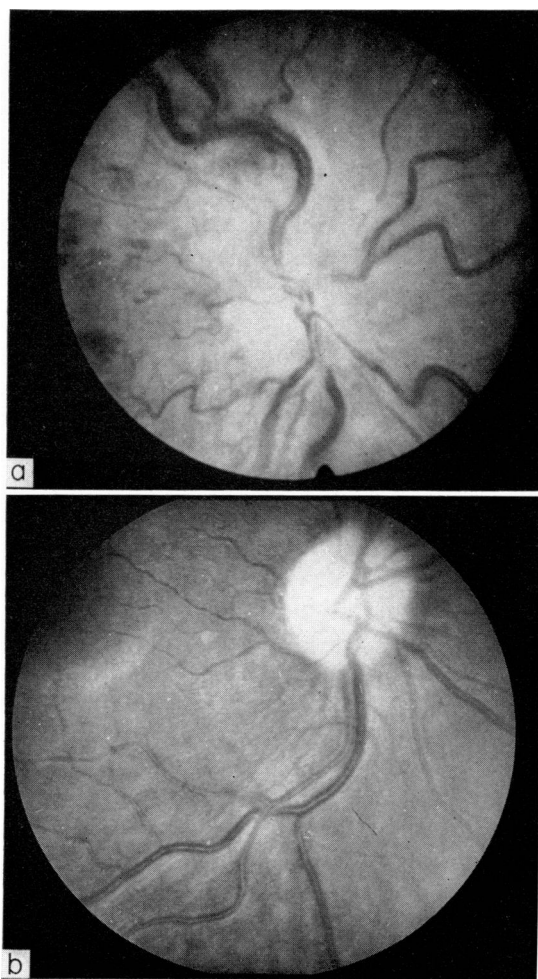


FIG. 4. Effects of plasma exchange on the fundi of an IgG myeloma patient. (a) Pre-exchange: note distended tortuous veins, papilloedema and haemorrhages. (b) Post-exchange: note near normalization.

who was semiconscious and bleeding from hyperviscosity manifestations recovered consciousness and stopped bleeding while plasma exchanges were in process (Powles *et al.*, 1971b) and we have had similar experience of this in a 65-yr-old female with an IgA myeloma and hyperviscosity syndrome. A patient who had Waldenström's macroglobulinaemia, who was semicomatose on arrival, responded within 6 hr to a 6-l plasma exchange.

Another patient with a malignant IgG2K monoclonal crystalline cryoglobulinaemia who developed repeated attacks of pseudobacterial endocarditis, pneumonia and other infections, responded to exchange treatment. *In vitro*, the abnormal cryoglobulin had an inhibitory effect on *Candida* killing and this was corrected in his post-exchange plasma.

Patients with myeloma, who develop renal failure, have a poor prognosis. Often the blood urea is reduced following exchanges. As renal failure in myeloma may be due to a variety of reasons, more detailed studies of renal function before and after plasma exchanges will be necessary before beneficial claims can be established for this form of treatment. A 43-yr-old Jamaican female patient with IgG2L myelomatosis resistant to Melphalan, developed severe hyperviscosity (Fig. 4a) and malignant hypertension (b.p. 170/140); relative plasma viscosity was 700 units (normal range 150–180); total protein was 180 g/l; paraprotein 149 g/l; Bence-Jones proteinuria; blood urea 20 mg/100 ml, with normal values for electrolytes. Emergency plasma exchanges consisted of two of 5 l and 6 l over 48 hr. Anti-hypertensive agents—bethanidine, methyl DOPA were included in her treatment. After six repeated plasma exchanges over 10 weeks, her viscosity was reduced to near normal, serum protein to 89 g/l and her hypertension became controlled without anti-hypertensive agents. At 20 months she is well with normal fundi (Fig. 4b), creatinine clearance 120 ml/min, b.p. 120/90, total serum protein 90 g/l and plasma viscosity 300 units. She is now on cyclical chemotherapy of CCNU, cyclophosphamide, prednisone and melphalan, with plasma exchange once monthly.

Removing 'blocking factors' during cancer growth. Both in human and experimental animals, circulating inhibitory factors are present in the host serum, which impair the host's ability to reject growing tumours (Hellström, Hellström & Sjögren, 1971). Some of these have been identified as possible soluble tumour antigen-antibody complexes, excess tumour antigens or possibly useless immunoglobulins (? IgG2). In some of these situations, washing the lymphocytes repeatedly has improved tumour kill (Currie *et al.*, 1972). In other situations, reduction of host serum to less than 20% in a mixed lymphocyte reaction can often remove 'blocking activity' (Butterworth *et al.*, 1974). Since this is achievable, whereas less than 10% is not, we use this *in vitro* test to judge whether or not to attempt plasma exchange. Fig. 5 shows the amazing reduction in VMA output when anti-tumour treatment, previously with little effect, was given immediately after an efficient plasma exchange (which was shown *in vitro* to abolish 'blocking'). Immune complexes may be detectable in these cancer patients by examining their sera by ultracentrifugation, or for their capacity to produce complement *in vitro* activation (CIA test of Versey, in preparation). The affinity of the tumour antigen to antibodies can then be studied so that after dissociation 'pure' tumour antigen and 'tumour-specific antibodies' can be obtained for therapeutic uses.

Harvesting of antibodies. Antibodies against micro-organisms, Rhesus factor or those created in cancer patients by homologous immunization, can be of therapeutic use. Human anti-serum against tetanus toxoid has replaced horse antisera (McComb & Dwyer, 1963), anti-D is used to prevent sensitization of Rhesus-negative mothers, and hyperimmune sera against *Pseudomonas pyocyaneus* have been raised in volunteers and used to treat infected burns (Feller & Pierson, 1968). In two children with extensive metastatic neuroblastoma, we

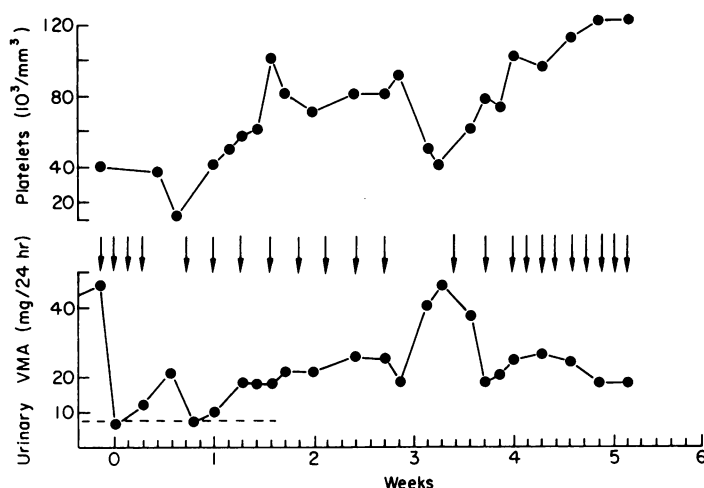


FIG. 5. Effects of 'unblocking' a boy with disseminated neuroblastoma (just before 0) so that treatment with homologous immune serum, chlorambucil-treated, dramatically reduced the VMA output (much more and for longer than the actual plasma volume washout), presumably by reducing the tumour mass, as also evidenced by the platelet recovery. The dashed line signifies the normal upper limit.

have found that the antineuroblastoma antibodies created by homologous immunization, when previously treated with chlorambucil, were more cytotoxic to the tumour cells than antibodies or chlorambucil alone (Oon *et al.*, 1974). A beneficial response was seen, where chemotherapy and/or radiotherapy were ineffective in controlling the disease. One such child, who failed to respond to full doses of adriamycin and radiotherapy, rapidly improved when given human antineuroblastoma antibody, freshly treated with chlorambucil simultaneously. An enhanced cytotoxic killing of these cells was seen *in vitro*, and there was remarkable radiological improvement clinically and in her chest X-ray (Fig. 6).

Treatment for hepatic failure. On many occasions, hepatic failure due to viral hepatitis and/or cirrhosis, has been treated using the CFC. Graw, Buckner & Eisel (1969) reported biochemical improvement in serum bilirubin, alkaline phosphatase, SGPT, SGOT in both the patients with viral hepatitis and cirrhosis; confirmed in one of our own patients with chronic aggressive hepatitis. This, however, may not in any way alter the prognosis of the underlying disease (Redekar & Yamahiro, 1973). The only indication for plasma exchange in hepatic failure would appear to be limited to those conditions which are potentially reversible, e.g. toxic hepato-cellular failure, or supporting hepatic transplants.

Removal of autoantibodies. Temporary removal of autoantibodies causing symptoms may be important. Laventhal (personal communication) treated a patient with a retropharyngeal haematoma who had antibodies against antihæmophilic factor (Factor VIII), by plasma exchange with fresh frozen plasma and then replacing the Factor VIII deficiency by concentrates of cryoprecipitate.

In a patient with rheumatoid arthritis and hyperviscosity, we removed about 80% of the circulating rheumatoid factor by plasma exchange (Table 3). Another patient with a lymphoma of the small bowel, IgM paraproteinaemia, hyperviscosity and generalized autoantibodies (including those reacting against autonomic and peripheral neurones) improved

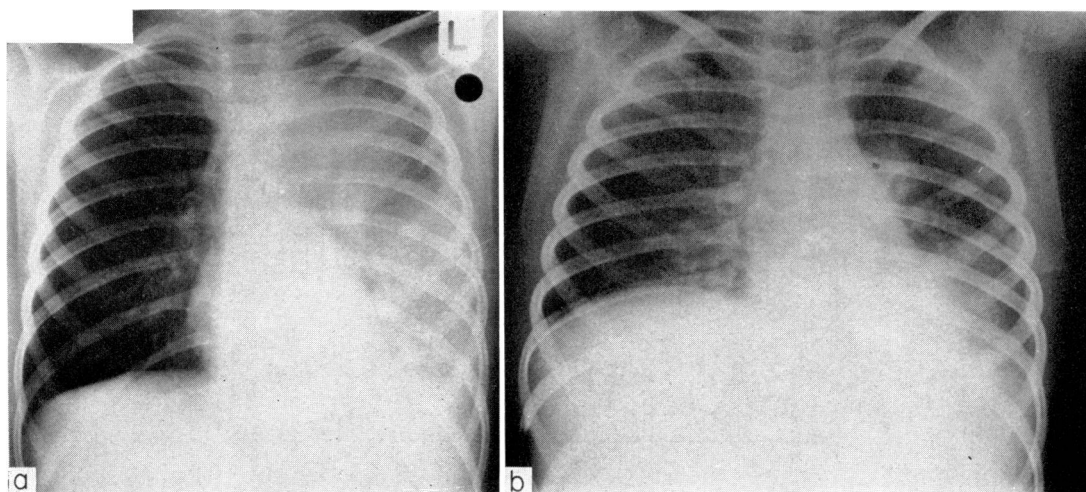


FIG. 6. A marked reduction of neuroblastoma metastases in the left lung from (a) to (b), by use of chlorambucil-treated IgG, prepared from harvested homologous immune serum.

after exchange with plasma. It could also be predicted that an acute exacerbation of immune complex disease (DLE) or serious mixed cryoglobulinaemia could be helped by an efficient plasma exchange.

The benefits are achieved rapidly, and then specific chemotherapy can be given to reduce the synthesis or effects of these abnormal globulins.

(C) COMPLICATIONS

Table 4 summarizes complications which can occur with the use of the CFC, and their prophylaxis and management are considered below.

Hypocalcaemia

Hypocalcaemia with and without serious sequelae may develop during routine leuka-phoresis, plasmaphoresis or erythrophoresis. This may be the result of removal of a large amount of ionizable calcium. To avoid this danger, the machine should be primed with Hartmann's or any other physiological solution containing calcium. Cardiac arrhythmias, such as nodal, atrial and ventricular ectopics and sinus bradycardia not infrequently occur, even though no clinical symptoms are apparent. These arrhythmias are seen when plasma calcium levels fall to below 8.5 mg%. The development of severe sinus bradycardia, going on to atrio-ventricular dissociation and hypotension may occur rapidly, unless this cause is recognized early and reversed rapidly by administering 10 ml of 10% calcium gluconate and 0.6 mg of atropine intravenously. With the use of acid-citrate-dextrose as anticoagulant in dry reconstituted and fresh frozen plasma, more marked lowering of serum calcium occurs. Many of the symptoms of hypocalcaemia such as parasthesia, cramps and electrocardiographic changes may be avoided by giving prophylactic calcium (as 10% calcium gluconate) as each 1.5 l of exchange is reached or even earlier, if a preliminary serum calcium suggests

TABLE 3. Pre- and post-exchange values in a female of 24 years with Sjögren's syndrome and rheumatoid arthritis who had a 5 l exchange

	Pre-exchange	Post-exchange
Plasma viscosity (normal range 150–175 u)	236	170
Rheumatoid Factor (Latex flocculation)	1:2048	1:512
Serum levels in g/l		
IgG	28	17.6
IgA	17.60	7.4
IgM	1.55	1.5
Total protein	96	73

TABLE 4. Medical problems associated with the use of the continuous flow centrifuge

Venospasm
Chills*
Pyrexia*
Hypothermic reactions
Hypocalcaemia
IgM isohaemagglutinins, haemolysis
Thrombocytopenia
Bleeding
Transfusion hepatitis
Cardiac stress, arrhythmias
Asymptomatic hypotension

* Immunological (type I, urticaria, wheezing, etc.) reactions to exchange materials due to inborn 'absence' of proteins (e.g. IgA deficiency) or prior sensitization to altered proteins.

a low ionized level. Magnesium blood levels do fall, but they are not sufficiently low to require intravenous replacement. Similarly, other electrolytes are not seriously affected.

Syncope

A few young, healthy apprehensive individuals sometimes faint from a vasovagal reaction. Where such a previous history obtains, it has been advantageous to give them a premedication of atropine 0.6 mg. i.m. shortly before the run. Syncope occurring during plasma exchange is usually associated with severe sinus bradycardia and hypotension. In most instances, these effects are due to hypocalcaemia (v.s.).

Hypotension

Asymptomatic hypotension occasionally occurs when patients with hyperviscosity from Waldenstrom's macroglobulinaemia myeloma are plasma-exchanged. The systolic pressure

may fall by 20–30 mm in less than 30 min. This phenomenon is seen in new patients who have not experienced plasmaphoresis before, and may reflect the inability of the patient's blood vessels to compensate rapidly to the changes of intravascular plasma volume. These effects may be rectified by allowing the patients to be slightly more in positive fluid balance. When patients with cryoglobulinaemia, which precipitate at room temperature (21°C) are treated, symptomless hypotension may appear when the room is warmed up to 34°C. This may reflect pooling of blood in the peripheries, as the cutaneous vessels vasodilate to the heat. On cooling the room at the end of the run, the blood pressure reverts to normal levels.

Cardiac failure

Cardiac failure may be precipitated if elderly patients are infused too rapidly with fluids, and this may occur at the end of the run, when they are allowed to have the rest of the blood in the machine (amounting to about 500 ml). It would, therefore, be judicious to put such patients into negative fluid balance, before returning the rest of the fluid in the machine, so that they remain in balance at the end.

Cardiac arrhythmias

Patients with coexisting atrial fibrillation, or who have been on digoxin or beta-blockers, are not adversely affected by leukaphoresis or plasmaphoresis. However, patients with ventricular ectopics are more sensitive to the effects of hypocalcaemia. Intravenous calcium gluconate and lignocaine are very useful for reverting such arrhythmias.

Patients who have recently been, or are on, monoamine oxidase inhibitors, should avoid having plasma exchange or any of these procedures. They are likely to develop severe hypotensive episodes.

Immunological reactions to plasma

Allergic reactions to transfused plasma, e.g. chills, urticaria, wheezing, rigors, may occur in patients who are sensitive to certain antigens in such plasma. Such patients may have multiple leucocyte antibodies and the use of suitable single donor plasma would be indicated. Similarly in patients with severe IgA deficiency, anaphylaxis (Amman & Hong, 1971) may occur when normal plasma is transfused. Autoimmune diseases are known to have up to a twenty-fold increased incidence of IgA deficiency so such patients should be adequately screened and recognized before plasma exchange is done.

IgM isohaemagglutinins

Where large volumes of plasma are required for plasma exchanges, it is important that the correct blood group should be used.

However, pooled plasma is more readily available than fresh frozen plasma, and contains donors of different blood groups. It is therefore important that such pooled plasma should contain no excess isohaemagglutinins, as they may give rise to haemolysis following transfusion (Wood, Price & Childes, 1967).

Thrombocytopenia

Patients who suffer from hyperviscosity or cryoglobulinaemia may develop transient thrombocytopenia following removal of their viscous plasma. Protamine may be necessary, should there be any evidence of actual bleeding, but more often bleeding from hyperviscous states improves following plasmaphoresis.

Infection

The danger of transferring infections from blood donors to recipients is a real one. However, in our own experience, we have not encountered transferred bacterial infections, even though many of these patients suffered from severe immune deficiency states, or defects in phagocytosis. Nevertheless, scrupulous aseptic techniques should be practised throughout and random spot checks during the run would help to maintain aseptic standards.

Hypothermic reactions

The use of large volumes of intravenous fluids, during plasma exchanges, at room temperatures (22–24°C), has been responsible for reactions such as shivering, rigors or 'hypertensive episodes'. These occur mainly in long runs of 4–6 hr. One of the reasons for this cold fluid infusion, is due to cooling of blood from the bowl, for example: temperature of donor's blood entering bowl = 38.4°C; temperature of donor's packed red cells, from red cell line = 26°C ± 2°C; temperature of donor's plasma from plasma line = 26°C ± 2°C; temperature of blood entering donor = 24°C ± 2°C.

This problem has been overcome by using a thermostatically controlled Portex (Mark II) Blood Warmer, into the returning line. Temperatures of such returning infusion fluids are 35°C ± 2°C.

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